Journal of the Saudi Society of Agricultural Sciences (2015) **xxx**, xxx–xxx

King Saud University

Journal of the Saudi Society of Agricultural Sciences

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Influence of salicylic acid on seed germination of *Vicia faba* L. under salt stress

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Received 7 August 2015; revised 12 October 2015; accepted 13 October 2015

KEYWORDS

Salt stress;
Salicylic acid;
Germination percentage;
Germinated seeds fresh and dry weight;
Vicia faba L.

Abstract Seed germination is the critical stage for species survival. Salinity affects germination and seedling growth and yield of several crop species, such as broad bean. That is why this study was carried to evaluate the effects of NaCl on seed germination and influence of salicylic acid on seed in order to improving salt tolerant on broad bean. *Vicia faba* L. is an important pulse crop in the Mediterranean region. In many cases broad bean is grown on saline soils where growth and yield are limited by salinity. The results showed that Irrigation with saline water significantly reduced all seed germination parameters in comparison with the respective control. Alleviation of growth arrest was observed with exogenous applications of salicylic acid (SA) under salt stress conditions. Overall, the positive effect of SA towards resistance to the salinity of *V. faba* L. will provide some practical basis for *V. faba* L cultivation.

1. Introduction

There has been a renewed interest in broad bean (*Vicia faba* L.) throughout the world, which may not be unconnected with its high protein content, rising costs of protein-rich food and feed, national desires for greater self sufficiency in food production and perhaps most importantly, the rapidly increasing human populations (Gueguen and Cerletti, 1994). In Morocco the most cultivated legume is the broad bean (*V. faba* L.), and represents almost half of the sole and the total production of pulses (ONICL, 2013). Nevertheless, climate change has become major constraints to agricultural production in Morocco. Salinity affects large areas of Moroccan soils especially in semi-arid and irrigated areas (Drevon and Sifi, 2003). However, the adaptation to the salinity during seed germination and seedling growth is very important for plant growth, whereas Seed germination is a mechanism, in which morphological and physiological alterations result in activation of the embryo. Before germination, seed absorbs water, resulting in the expansion and elongation of seed embryo. When the radicle has grown out of the covering seed layers, the process of seed germination is completed (Hermann et al., 2007). The period of germination and establishment is the most critical stage in the life cycle, which is a crucial factor in determining the species distribution and community components, and seeds of most plant species have the highest resistance to extreme environmental stresses (Gutterman, 1993). Many researchers have evaluated the processes involved

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Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jssas.2015.10.002

in seed germination, and how they are affected by abiotic stress. Saline habitat is one kind of the most stressful habitats which is known to affect many physiological and ecological characteristics, such as plant growth, development, reproduction and geographical distribution (Qu and Huang, 2005). The available literature revealed the effects of salinity on the seed germination of various crops such as *Oryza sativa* (Xu et al., 2011), *Triticum aestivum* (Akbarimoghaddam et al., 2011), *Vigna* spp. (Jabeen et al., 2003), and *Helianthus annuus* (Mutlu and Buzcuk, 2007). It is well established that salt stress has negative correlation with seed germination (Anaya et al., 2013). Higher level of salt stress inhibits the germination of seeds while lower level of salinity induces a state of dormancy (Khan and Weber, 2008). Increasing salinity leads to a reduction and/or delay in germination of plants and death of seeds before germination (Song et al., 2005).

Many researchers have evaluated the processes involved in seed germination, and how they are affected by plant hormones in a range of plant families (Hermann et al., 2007). However, salicylic acid (SA) as one of the potential endogenous plant hormones plays an important role in plant growth and development, and this actual role in abiotic stresses remains unresolved. Several methods of application (soaking the seeds prior to sowing, adding to the hydroponic solution, irrigating, or spraying with SA solution) have been shown to protect various plant species against abiotic stress by inducing a wide range of processes involved in stress tolerance mechanisms (Horváth et al., 2007). But to our knowledge there is no study on the effect of SA on the broad bean germination. This study was carried out to obtain information on the effect of salinity (NaCl), salicylic acid (SA) and combined treatment NaCl/SA on the germination. The objective of this research was to study the effect of salt stress and salicylic acid on some characteristics of *V. faba* by measuring seed germination at various concentrations of NaCl (0, 90, 120, 150 and 200 mM) and four levels of salicylic acid (0, 0.25, 0.5 and 1 mM) in order to improving salt tolerant on broad bean.

2. Materials and methods

2.1. Plant material

The study was conducted in the laboratory conditions, to determine the effects of salinity and salicylic acid on germination of *V. faba* L. Reina Mora cv.

2.2. Seed germination experimental design

Broad bean (*V. faba* L.) seeds were selected for uniformity by choosing those homogeneous and identical in size and colour, and free from wrinkles. The seeds were disinfected by sodium hypochlorite (1%) for 10 min, and then rinsed with distilled water to remove all traces of chlorine. However, only healthy seeds are selected for germination tests. Afterwards, seeds were soaked in distilled water for 8 h. The hydrated seeds were placed on Petri dishes. The seeds (4 replicates of 10 seeds) were germinated in darkness at 22 °C for 7 days.

Different concentrations of NaCl and SA were prepared. For each concentration of NaCl (0, 90, 120, 150 and 200 mM), germination tests were carried out at different

Table 1 Experimental design of different treatment combination.

NaCl (mM)	0				90				120				150				200			
SA (mM)	0	0.25	0.5	1	0	0.25	0.5	1	0	0.25	0.5	1	0	0.25	0.5	1	0	0.25	0.5	1
Treatment NaCl/SA	CONTROL	0/0.25	0/0.5	0/1	90/0	90/0.25	90/0.5	90/1	120/0	120/0.25	120/0.5	120/1	150/0	150/0.25	150/0.5	150/1	200/0	200/0.25	200/0.5	200/1

concentrations of salicylic acid (0, 0.25, 0.5 and 1 mM SA) in the dark at 22 °C (Table 1).

Treatments were assessed in factorial experimental based on a completely randomized design at 4 replications. Each replication includes one Petri dish (ten seeds per Petri dish).

The germination process was evaluated during 7 days; germinated seeds were counted every day. Seeds were considered to have germinated when radical had emerged and elongated by at least 2 mm.

Different characteristics of germination were determined:

1. Germination percentage (%) = $n/N \times 100$ with n: number of germinated seeds on the nth day and N: total number of seeds
2. Germination index (GI) according to the equation of Kader and Jutzi (2004). GI = Σ(TiNi). Ti: number of day after sowing and Ni: number of germinated seeds in the day.
3. Precocity of germination: The precocity of seeding which corresponds to the rate of seeds germinated from the 1st day.
4. Total germination (TG) measured in the seventh day using the formula TG (%) = (total number of germinated seeds/total seed) $\times$ 100.
5. Mean germination time (MGT) calculated according the formula of Ellis and Roberts (1981). MGT = Σ(ni/di). With ni: number of germinated seeds and di: day of counting.
6. Germinated seeds fresh and dry weight.

3. Results and discussion

3.1. Effect of salicylic acid on seed germination percentage

Statistical analysis (Table 2) shows a high significant effect of NaCl and salicylic acid on the germination of *Vica faba* L. (Reina Mora cv.) seeds. The effect of NaCl concentration on the percentage of germination in the different treatments during 7 days is shown in Fig. 1. Seeds start germination after one day (30%) and the maximum rate of germination (80%) is reached after 5 days under control treatment. In the presence of SA (0.25, 0.5 or 1 mM), seeds germination started also at the first day but with a lake percentage (lower than 20% in all SA treatments) to achieve the maximum of 70% with 0.25 mM and 0.5 mM SA after 5 days (Fig. 1a). High significance effect ($p < 0.001$) of salt stress is revealed, where germination process was modified by delaying and decreasing germination percentage. The maximum of germination was in decreasing with increasing salt stress to reach 70%, 62%; 50% and 45% respectively under 90, 120, 150 and 200 mM of NaCl in the sixth day (Fig. 1b). Many authors demonstrated the same effect of salt stress in different species (Orlovsky et al., 2011; Chang et al., 2010). Chang et al. (2010) demonstrated that the inhibition of seed germination induced by salt stress was related to the suppression of ethylene production during imbibitions.

Table 2 ANOVA table summarizing two-way completely randomized effects of treatment salinity, salicylic acid and their interactions on germination percentage and germination index of *V. faba* seeds.

	Df	Germination percentage	Germination index
SA	3	44.896018***	3.3088737 ns
Times	6	27.870122***	299.87505***
Times * SA	18	9.3325452***	5.8322588***
Salinity	4	6906.5942***	26.74614***
Salinity * SA	12	43.546923***	5.5297883***
Salinity * Times	24	24.525115***	6.411088***
Salinity * SA * Times	72	9.2873849***	3.0420747***

ns = not significant.
* difference statistically significant at $p < 0.05$.
** difference statistically significant at $p < 0.01$.
*** difference statistically significant at $p < 0.001$.

Nevertheless, the combination of SA with salinity had a benefic effect on the germination, with high significance effect ($p < 0.001$). Comparison between the respective treatments of salinity with and without SA, shows that germination percentage increased with increasing SA concentrations. After 3 days the best germination percentage was obtained at 0.25 mM SA under different concentrations of NaCl (90, 120, 150 and 200 mM) to reach 78%, 70%, 50% and 35% respectively (Fig. 1c). Even so, under higher concentration the increasing of SA dose increases remarkably the germination to reach 70% and 65% respectively for 150 and 200 mM NaCl (Fig. 1e). SA had an effective effect in alleviating the inhibitory effect of salt stress on seed germination, where, Primed seeds had better emergence percentage in salt stress in comparison with un-primed seeds. It is obvious that metabolic activities in primed seeds during germination process commenced much earlier than radicle and plumule appearance, so primed seeds emerged earlier than non-primed ones (Hopper et al., 1979).

3.2. Effects of SA, NaCl and SA/NaCl on germination index of *V. faba* L.

Fig. 2 shows the germination index of the *V. faba* seeds during 7 days at the five salinity levels (0, 90, 120, 150 and 200 mM) with or without salicylic acid.

There were significant differences in the germination index between treatments for all salinity levels. Statistical analysis (Table 2) displayed that salicylic acid alone has not any effect on the germination index. However, this parameter was significantly affected ($p < 0.001$) by different salt stress levels and by the combination of this one with all other factors (SA and times). The germination index under control treatment reached a maximum of 1.2 seed/day after 6 days. Nevertheless the germination index (GI) decreased with the increase in salt stress concentration compared to control to attain 1, 0.9, 0.7 and 0.6 seed/day in 90, 120, 150 and 200 mM NaCl respectively. However, the combination with SA improves GI to attain 1.1 and 1 seed/day respectively under 90 and 120 mM NaCl combined with 0.25 mM of SA. Arteca (1995), reports that SA induces and maximizes germination in lower concentrations and shows a preventing influence in higher concentrations. When SA was applied the emergence index was reduced due to the increase in the concentration of SA. Increasing SA concentration caused to enhance ABA synthesis, which can stop the seed germination (Wu et al., 1998).

3.3. Measure of the precocity of germination on broad bean (*V. faba L.*) under salt stress

The evolution of the early seed germination according to different concentrations of NaCl or/and SA is shown in Fig. 3.

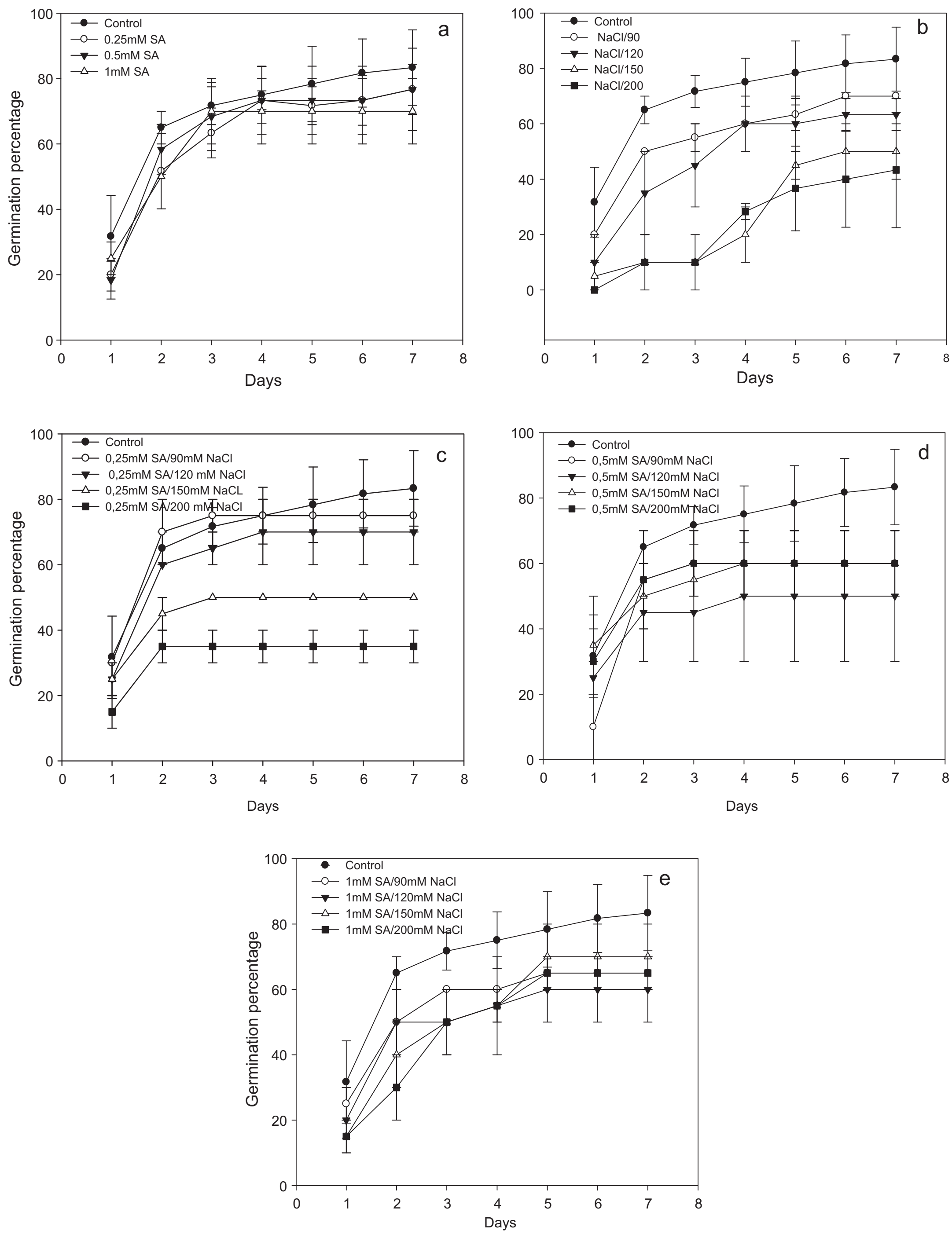

Figure 1 Germination percentage of *Vicia faba* seeds in different concentrations of salicylic acid (SA mM; a) and salt stress (NaCl mM; b) and salt stress combined with different SA concentrations 0.25 mM (c), 0.5 mM (d) and 1 mM (e), after 7 days at 28 °C in darkness. Data are the means (±S.E) of three replicates.

Germination precocity was retarded in comparison with the control with increasing SA concentration and salinity levels. However, statistical analysis reveals that salinity alone doesn't affect significantly germination precocity. In contrast, SA and the interaction between SA and salinity had a higher significant ($p < 0.001$) effect on germination precocity (Table 3), where the combination of both this factors (SA and salinity) improves the precocity under all salinity levels especially under 0.5 mM of SA, which reach 20%, 18% and 25% respectively to 120, 150 and 200 mM NaCl. Precocious germination was decreased with increasing NaCl salinity. However, the *V. faba* precocious germination at different salinity concentrations was increased significantly with SA application.

3.4. Total germination TG

Fig. 4 shows the effect of SA on total germination at different salinity concentrations. The total germination decreased with

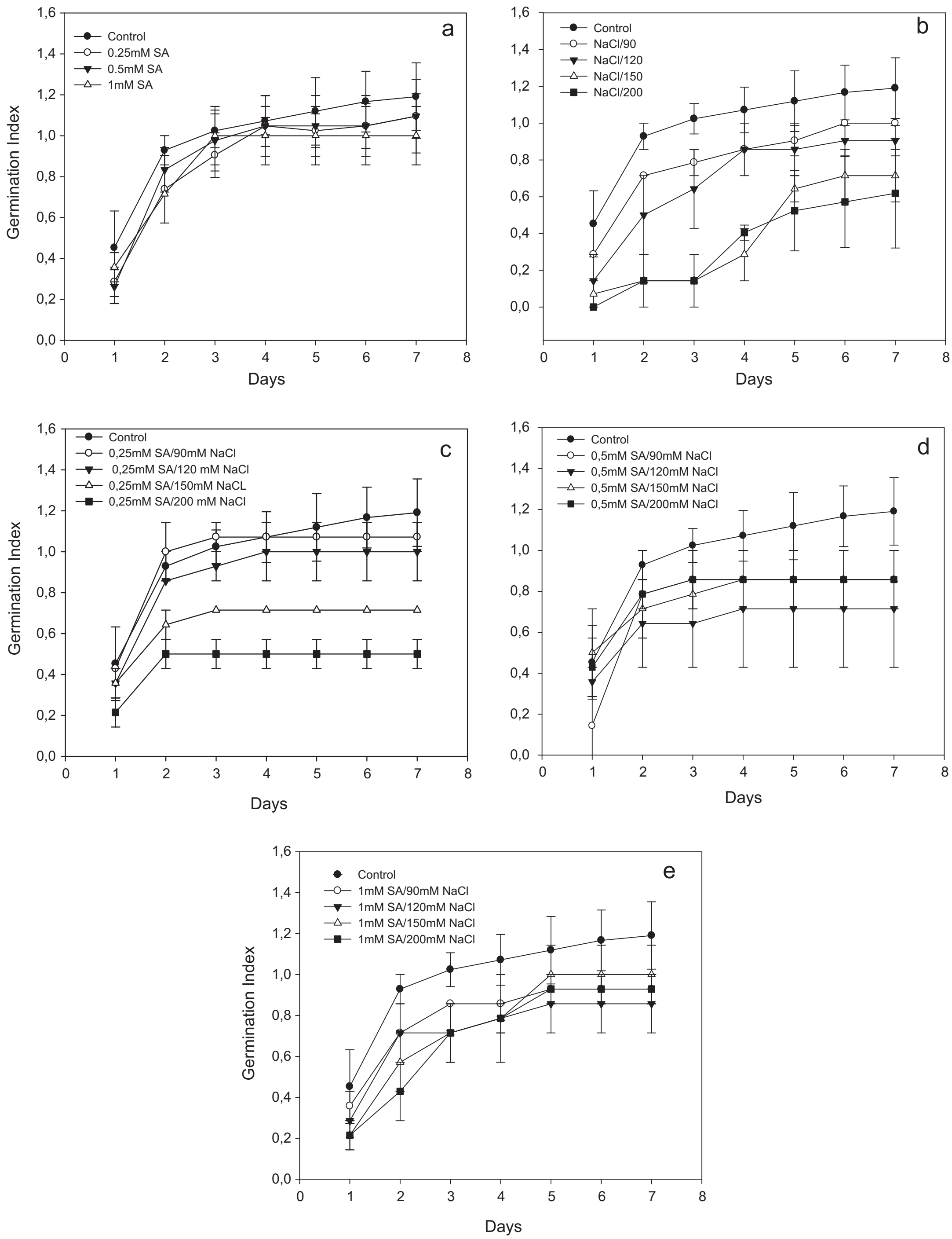

Figure 2 Germination index (% ±S.E.) of *Vicia faba* seeds in different concentrations of salicylic acid (SA mM; a) and salt stress (NaCl mM; b) and salt stress combined with different concentrations of SA 0.25 mM (c), 0.5 mM (d) and 1 mM (e), after 7 days at 28 °C in darkness.

increasing NaCl salinity. However, faced the different concentrations of NaCl, we notice two groups: the first group (90 mM and 120 mM NaCl) is characterized by a slight increase with 0.25 mM SA of the total germination against a decrease with 0.5 and 1 mM SA. The second group (150 and 200 mM NaCl) is characterized by an improvement in the total germination in the presence of 0.5 and 1 mM SA, which reaches up to 70% and 65% respectively to 150 mM and 200 mM combined with 1 mM SA. Priming causes some physiological changes including the sugar content, organic compounds and cumulated ions in the seed, root and finally the plant leaves leading to high rate of germination and more resistance to inclement conditions (Alvarado et al., 1987). Madah (2005) reported that low concentrations of salicylic acid increased the germination

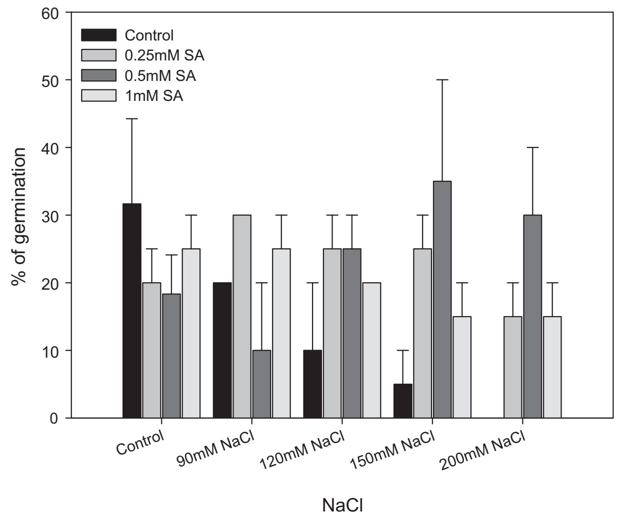

Figure 3 Precocious germination (% ±S.E.) of *Vicia faba* seeds in different concentrations of salicylic acid (SA mM; a) and salt stress (NaCl mM; b) and salt stress combined with different concentrations of SA 0.25 mM (c), 0.5 mM (d) and 1 mM (e), after 7 days at 28 °C in darkness.

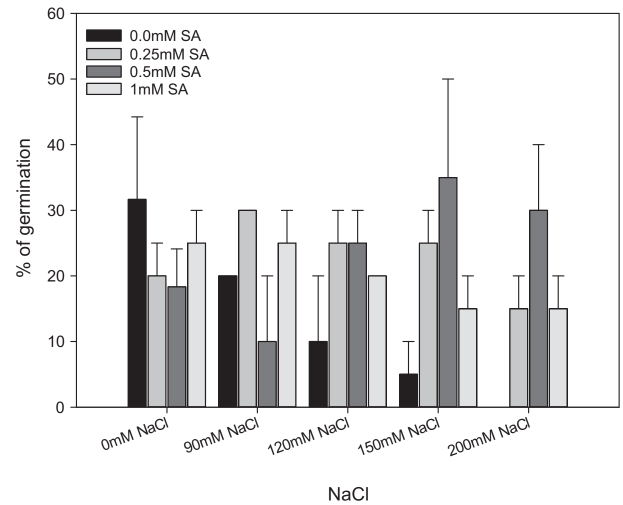

Figure 4 Total germination of *Vicia faba* seeds in different concentrations of salicylic acid (SA mM) and salt stress (NaCl mM) and salt stress combined with different concentrations of SA.

percentage, but this increase was not significant compared to control treatment.

3.5. Mean germination time (MGT)

Fig. 5 illustrates that for all treatment, increasing the NaCl concentration causes a decrease of the mean germination time (MGT). The results show that lowest SA concentration (0.25 mM) improves MGT with 90 mM and 120 mM but not with higher concentration of NaCl (150 mM and 200 mM). Nevertheless, with 0.5 mM and 1 mM of SA, the MGT increased with higher concentration (150 and 200 mM of NaCl) but decreased with lowest concentration (90 and 120 mM). According to Reinhardt and Rost (1995), most plants are more sensitive to salinity during germination and seedling growth. This is in agreement with our study. According to Nun et al. (2003) salicylic acid can inhibit the activity of catalase. Reduction of catalase activity leading to increased hydrogen peroxide that can improve some seeds germination. It is possible that SA stimulates the seed germination via

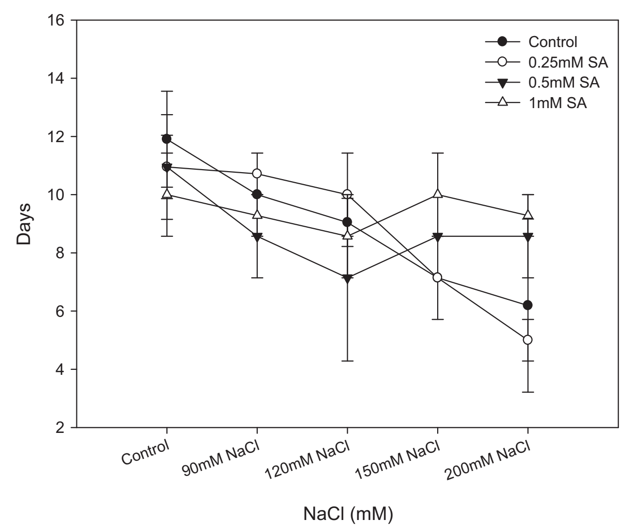

Figure 5 Mean daily germination of *Vicia faba* seeds in different concentrations of salicylic acid (SA mM) and salt stress (NaCl mM) and salt stress combined with different concentrations of SA.

Table 3 ANOVA table summarizing two-way completely randomized effects of treatment salinity, salicylic acid and their interactions on precocious germination (Pg), fresh weight (FW), dry weight (DW), total germination (TG) and mean daily germination (MDG) of *V. faba* seeds.

	Salicylic	Traitement	Salicyliq * Traitement
Df	3	4	12
Pg	6.9195402***	2.5215517 ns	5.6479885***
FW	1.8160291 ns	15.664421***	0.0087566 ns
DW	4.3030939*	13.060523***	6.878537***
TG	0.7493113 ns	11.586777***	3.0082645**
MDG	0.7493113 ns	11.586777***	3.0082647**

ns = not significant.
* difference statistically significant at $p < 0.05$.
** difference statistically significant at $p < 0.01$.
*** difference statistically significant at $p < 0.001$.

bio-synthesis of gibberellic acid and acts as thermogene inducers (Shah, 2003).

3.6. Germinated seeds fresh and dry weight

Fresh and dry weights of seedling of *V. faba* grown in different concentrations of NaCl with or without SA are presented in Fig. 6. Fresh weight of seedling significantly decreased due to the increase in the salinity compared to control (Fig. 4). Elsewhere, the application of SA improved the fresh weight compared to the respective salinity treatment especially with the application of 0.5 mM of SA. Though, at the different salicylic acid concentration the dry weight deceased significantly with increasing salinity levels ($p < 0.01$) compared to the control. However, the applications of SA improve the dry weight especially under 0.25 mM. Elsewhere, the salt stress and salicylic acid interaction on dry weight of *V. faba* were highly significant ($p < 0.001$) (Table 3). The same effect was reported by Entesari et al. (2012) on the mung bean grown under salinity stress and primed with SA. Also Baghizadeh and Hajmohammadrezaei (2011) indicated that drought stress caused reduction of fresh and dry weight of radical and plumule in comparison with seedling control of okra. However, the application of salicylic acid increases fresh and dry weight of radical and plumule.

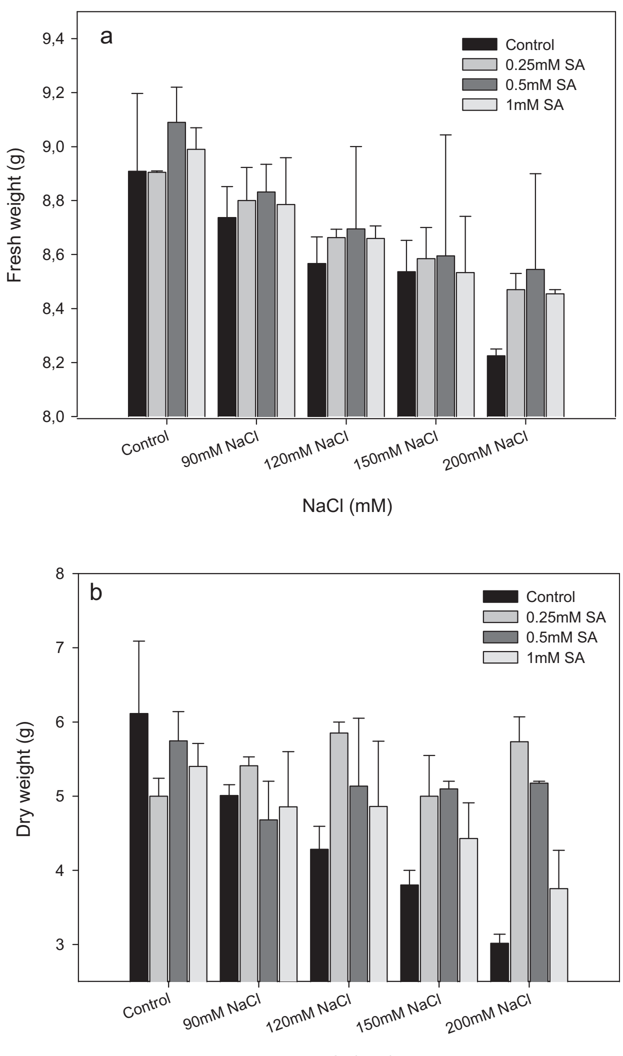

Figure 6 Fresh (a) and dry (b) weight (% ±S.E.) of *Vicia faba* seeds in different concentrations of salicylic acid (SA mM) and salt stress (NaCl mM) and salt stress combined with different concentrations of SA.

4. Conclusion

The overall results of this experiment showed the inhibitory effects of salt stress on seed germination parameters of broad bean (*V. faba* L.). However, seed priming with SA improved and enhanced germination traits under various stress levels, thus, reducing some of the inhibitory effects of salt stress. Based on the results, it can be concluded that low concentrations of SA have more positive effects on *V. faba* germination than medium and high concentrations. The 0.25 mM SA concentration significantly improved germination percentage and germination. This means that seed priming of *V. faba* with this low concentration of SA will speed up the germination time and enhance the establishment of seedlings. Therefore, the planted seeds will be less susceptible to soil-borne pests and diseases, and produce more biomass and photosynthetic capacity, especially in areas with saline irrigation water resources at planting stage. This has positive consequences on management of this crop at planting and germination stage. Based on the results of this study seed priming of *V. faba* with low concentrations of SA (0.25 mM) is recommended under low to high salinity stress conditions.

Conflict of interest

There is no conflict of interest.

References

Akbarimoghaddam, H., Galavi, M., Ghanbari, A., Panjehkeh, N., 2011. Salinity effects on seed germination and seedling growth of bread wheat cultivars. Trakia J. Sci. 9 (1), 43–50.

Alvarado, A.D., Bradford, K.J., Hewitt, J.D., 1987. Osmotic priming of tomato seed: effect on germination, field emergence, seedling growth and fruit yield. J. Am. Soc. Hort. Sci. 112, 427–432.

Anaya, F., Fghire, R., Issa Ali, O., Wahbi, S., Loutfi, K., 2013. Effet du stress salin sur la germination de fève (Vicia fab L.). 5ème Rencontre Nationale Gestion et Protection de l'Environnement G-ENVIRO5. 28-05-2013 Casablanca Maroc.

Arteca, R.N., 1995. Jasmonates, salicylic acid and brassinosteroids. In: Davies, P.J. (Ed.), Plant Hormones: Physiology, Biochemistry and Molecular Biology, second ed. Kluwer Academic, Publishers, pp. 206–213.

Baghizadeh, A., Hajmohammadrezaei, M., 2011. Effect of drought stress and its interaction with ascorbate and salicylic acid on okra (*Hibiscus Esculents* l.) germination and seedling growth. J. Stress Physiol. Biochem. 7 (1), 55–65.

Chang, C., Wang, B., Shi, L., Li, Y., Duo, L., Zhang, W., 2010. Alleviation of salt stress-induced inhibition of seed germination in cucumber (Cucumis sativus L.) by ethylene and glutamate. J. Plant Physiol. 167 (14), 1152–1156.

Drevon, J.J., Sifi, B., 2003. Fixation symbiotique de l'azote et développement durable dans le Bassin méditerranéen: Carthage, Tunisie, 13–16 octobre 1998 [et] Montpellier, France, 9–13 juillet 2000 (vol. 100). Editions Quae.

Ellis, R.H., Roberts, E.H., 1981. The quantification of ageing and survival in orthodox seeds. Seed Sci. Technol. (Neth.).

Entesari, M., Sharif-Zahed, F., Zare, S., Farhangfar, M., Dashtaki, M., 2012. Effects of seed priming on mung been (*Vigna radiate*) cultivars with salicylic acid and potassium nitrate under salinity stress. Int. J. Agric. Res. Rev. 2, 926–932.

Gueguen, J., Cerletti, P., 1994. Proteins of some legume seeds: soybean, pea, fababean and lupin. In: New and Developing Sources of Food Proteins. Springer, US, pp. 145–193.

Gutterman, Y., 1993. Seed Germination in Desert Plants. Springer-Verlag GmbH & Co, KG.

Hermann, K., Meinhard, J., Dobrev, P., Linkies, A., Pesek, B., Heß, B., Leubner-Metzger, G., 2007. 1-Aminocyclopropane-1-carboxylic acid and abscisic acid during the germination of sugar beet (*Beta vulgaris* L.): a comparative study of fruits and seeds. J. Exp. Bot. 58 (11), 3047–3060.

Hopper, N.W., Overholt, J.R., Martin, J.R., 1979. Effect of cultivar, temperature and seed size on the germination and emergence of soya beans (*Glycine max* (L.) Merr.). Ann. Bot. 44 (3), 301–308.

Horváth, E., Szalai, G., Janda, T., 2007. Induction of abiotic stress tolerance by salicylic acid signaling. J. Plant Growth Regul. 26 (3), 290–300.

Jabeen, M., Azim, F., Ibrar, M., Hussain, F., Ilahi, I., 2003. The effect of sodium chloride salinity on germination and productivity of Mung bean (Vigna mungo Linn.).

Kader, M.A., Jutzi, S.C., 2004. Effects of thermal and salt treatments during imbibition on germination and seedling growth of sorghum at 42/19 C. J. Agron. Crop Sci. 190 (1), 35–38.

Khan, M.A., Weber, D.J., 2008. Ecophysiology of High Salinity Tolerant Plants (Tasks for Vegetation Science), first ed. Springer, Amsterdam.

Madah, M., 2005. Effects of salicylic acid on some aspects of development, performance and resistance of chickpea (Cicer arientinum) in situ and in vitro conditions. Doctoral dissertation, Ph. D. dissertation. Islamic Azad University, Science and Research unit, Iran.

Mutlu, F., Buzcuk, S., 2007. Salinity induced changes of free and bound polyamine levels in sunflower (Helianthus annuus L.) roots differing in salt tolerance. Pak. J. Bot. 39 (4), 1097–1102.

Nun, N.B., Plakhine, D., Joel, D.M., Mayer, A.M., 2003. Changes in the activity of the alternative oxidase in Orobanche seeds during conditioning and their possible physiological function. Phytochemistry 64 (1), 235–241.

ONICL: Office national interprofessionnel des céréales et des légumineuses, bulletin d'information marché des légumineuses, 2013. <www.onicl.org.ma>.

Orlovsky, N.S., Japakova, U.N., Shulgina, I., Volis, S., 2011. Comparative study of seed germination and growth of Kochia prostrata and Kochia scoparia (Chenopodiaceae) under salinity. J. Arid Environ. 75 (6), 532–537.

Reinhardt, D.H., Rost, T.L., 1995. Primary and lateral root development of dark- and light-grown cotton seedlings under salinity stress. Bot. Acta 108, 403–465.

Qu, X.X., Huang, Z.Y., 2005. The adaptive strategies of halophyte seed germination. Acta Ecol. Sin. 25 (9), 2389–2398.

Shah, J., 2003. The salicylic acid loop in plant defense. Curr. Opin. Plant Biol. 6 (4), 365–371.

Song, J., Feng, G., Tian, C.Y., Zhang, F.S., 2005. Strategies for adaptation of Suaeda physophora Haloxylon ammodendron and Haloxylon ersicum a saline environment rigermination stage. Ann. Bot. 96, 399–405.

Wu, L., Guo, X., Harivandi, M.A., 1998. Allelopathic effects of phenolic acids detected in buffalograss (Buchloe dactyloides) clippings on growth of annual bluegrass (Poa annua) and buffalograss seedlings. Environ. Exp. Bot. 39 (2), 159–167.

Xu, X.Y., Fan, R., Zheng, R., Li, C.M., Yu, D.Y., 2011. Proteomic analysis of seed germination under salt stress in soybeans. J. Zhejiang Univ. Sci. B 12 (7), 507–517.

Please cite this article in press as: Anaya, F. et al., Influence of salicylic acid on seed germination of *Vicia faba* L. under salt stress. Journal of the Saudi Society of Agricultural Sciences (2015), http://dx.doi.org/10.1016/j.jssas.2015.10.002